

## **AMENDMENTS TO THE CLAIMS:**

This listing of claims will replace all prior versions and listings of claims in the application:

Claims 1-23. (Cancelled)

24. (Currently amended): A method for modifying a wild strain of an enteroinvasive *Shigella* to produce a modified strain of *Shigella* that can not spread substantially within infected cells of a host and can not spread substantially from infected to uninfected cells of the host, that can be used for use in making a vaccine against the wild strain of *Shigella*, the method comprising inactivating an *icsA* gene of the wild strain of *Shigella*, other than only by inactivation by means of a transposon inserted into the gene, to thereby provide a modified strain of *Shigella* that can not spread substantially within infected cells of the host and can not spread substantially from infected to uninfected cells of the host ~~so that it is defective in spread within infected cells and from infected to uninfected cells of the host.~~

25. (Currently amended): The method of claim 24, wherein the modified strain of *Shigella* also can not substantially invade cells of the host, the method further comprising inactivating an aerobactin or enterochelin gene of the wild strain of *Shigella*, other than only by inactivation by means of a transposon inserted into the gene, ~~so that it is defective in invading cells of the host~~ to thereby provide a modified strain of *Shigella* that can not spread substantially within infected cells of the host, can not spread

substantially from infected to uninfected cells of the host, and can not substantially invade cells of the host.

26. (Currently amended): The method of claim 25, wherein the modified strain of *Shigella* also can not produce toxins that kill a substantial number of the host's cells, the method further comprising inactivating a *Shiga*-toxin gene of the wild strain of *Shigella*, other than only by inactivation by means of a transposon inserted into the gene, to thereby provide a modified strain of *Shigella* that can not spread substantially within infected cells of the host, can not spread substantially from infected to uninfected cells of the host, can not substantially invade cells of the host, and can not produce toxins that kill a substantial number of the host's cells ~~so that it is defective in killing host cells.~~

27. (Previously presented): The method of any of claims 24-26, wherein said *Shigella* is *S. flexneri*.

28. (Previously presented): The method of any of claims 24-26, wherein said *Shigella* is *S. dysenteriae* 1.

29. (Previously presented): The method of claim 28, wherein one or more of the ent F, Fep E, Fep C, and Fep D subunit genes of the enterochelin operon of *S. dysenteriae* 1 are modified.

FINNEGAN  
HENDERSON  
FARABOW  
GARRETT &  
DUNNER LLP

1300 I Street, NW  
Washington, DC 20005  
202.408.4000  
Fax 202.408.4400  
www.finnegan.com

30. (Previously presented): The method of claim 26, wherein the *Shiga*-toxin gene is the *Shiga*-toxin A gene.

31. (Cancelled).

32. (Previously presented): The method of any of claims 24-26, wherein one or more of said inactivated genes are inactivated genes from which at least one nucleotide sequence has been deleted.

33. (Previously presented): The method of any of claims 24-26, wherein one or more of said inactivated genes are inactivated genes into which at least one nucleotide sequence has been inserted.

34. (Previously presented): The method of claim 33, wherein a marker gene is inserted into one or more of said inactivated genes.

35. (Currently amended): The method of claim ~~[[24]]~~ 26, further comprising isolating said modified strain of *Shigella* from said wild strain of *Shigella*.

36. (Currently amended): A modified *Shigella* for use in making a vaccine against a wild strain of *Shigella*, the modified *Shigella* comprising:

(a) an inactivated icsA gene, inactivated other than only by means of a transposon inserted into the gene; and

(b) an inactivated aerobactin or enterochelin gene, inactivated other than only by means of a transposon inserted into the gene;

wherein the modified *Shigella* can not spread substantially within infected cells of the host, can not spread substantially from infected to uninfected cells of the host, and can not substantially invade cells of the host.

37. (Currently amended): The *Shigella* of claim 36, further comprising an inactivated *Shiga*-toxin gene, inactivated other than only by means of a transposon inserted into the gene;

wherein the modified *Shigella* can not spread substantially within infected cells of the host, can not spread substantially from infected to uninfected cells of the host, can not substantially invade cells of the host, and can not produce toxins that kill a substantial number of the host's cells.

38. (Cancelled).

39. (Previously presented): The *Shigella* of claim 37, wherein the *Shiga*-toxin gene is *Shiga*-toxin A.

40. (Previously presented): The *Shigella* of claim 36 or 37, wherein said *Shigella* is *S. dysenteriae* 1 or *S. flexneri*.

41. (Previously presented): The *Shigella* of claim 36 or 37, comprising inactivated ent F, Fep E, Fep C, or Fep D subunit genes of the enterochelin operon.

42. (Cancelled).

43. (Previously presented): The *Shigella* of claim 36 or 37, wherein one or more of said inactivated genes are inactivated genes from which at least one nucleotide sequence has been deleted.

44. (Previously presented): The *Shigella* of claim 36 or 37, wherein one or more of said inactivated genes are inactivated genes into which at least one nucleotide sequence has been inserted.

45. (Previously presented): The *Shigella* of claim 44, wherein a marker gene is inserted into one or more of said inactivated genes.

46. (Previously presented): A vaccine comprising the *Shigella* of claim 36 or 37 and a pharmaceutically acceptable vehicle.

47. (Previously presented): The method of any of claims 24-26, wherein a marker gene is inserted into each inactivated gene.

FINNEGAN  
HENDERSON  
FARABOW  
GARRETT &  
DUNNER LLP

1300 I Street, NW  
Washington, DC 20005  
202.408.4000  
Fax 202.408.4400  
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48. (Previously presented): The *Shigella* of claim 36 or 37, wherein a marker gene is inserted into each inactivated gene.

49. (Previously presented): A vaccine comprising the *Shigella* of claim 48 and a pharmaceutically acceptable vehicle.

50. (Currently amended): ~~A method for modifying a wild strain of an enteroinvasive *Shigella* to produce a modified strain of *Shigella* that can be used for making a vaccine against the wild strain of *Shigella*, the method comprising inactivating an *icsA* gene of the wild strain of *Shigella* by allelic exchange with a mutagenized *icsA* gene that has been mutagenized *in vitro*, wherein said mutagenesis is other than only by means of a transposon inserted into the gene, so that the modified strain of *Shigella* is defective in spread within infected cells and from infected to uninfected cells of the host.~~

The method of claim 24, wherein said inactivation of said *icsA* gene comprises allelic exchange with a mutagenized *icsA* gene that has been mutagenized *in vitro*.

51. (Currently amended): ~~The method of claim 50, further comprising inactivating an aerobactin or enterochelin gene of the wild strain of *Shigella* by allelic exchange with a mutagenized aerobactin or enterochelin gene that has been mutagenized *in vitro*, wherein said mutagenesis is other than only by means of a transposon inserted into the gene, so that the modified strain of *Shigella* is defective in invading cells of a host.~~

FINNEGAN  
HENDERSON  
FARABOW  
GARRETT &  
DUNNER LLP

1300 I Street, NW  
Washington, DC 20005  
202.408.4000  
Fax 202.408.4400  
www.finnegan.com

The method of claim 25, wherein said inactivation of said *icsA* gene comprises allelic exchange with a mutagenized *icsA* gene that has been mutagenized *in vitro*, and wherein said inactivation of said aerobactin or enterochelin gene comprises allelic exchange with a mutagenized aerobactin or enterochelin gene that has been mutagenized *in vitro*.

52. (Currently amended): ~~The method of claim 51, further comprising inactivating a *Shiga*-toxin gene of the wild strain of *Shigella* by allelic exchange with a mutagenized *Shiga*-toxin gene that has been mutagenized *in vitro*, wherein said mutagenesis is other than only by means of a transposon inserted into the gene, so that the modified strain of *Shigella* is defective in killing host cells.~~

The method of claim 26, wherein said inactivation of said *icsA* gene comprises allelic exchange with a mutagenized *icsA* gene that has been mutagenized *in vitro*, wherein said inactivation of said aerobactin or enterochelin gene comprises allelic exchange with a mutagenized aerobactin or enterochelin gene that has been mutagenized *in vitro*, and wherein said inactivation of said *Shiga*-toxin gene comprises allelic exchange with a mutagenized *Shiga*-toxin gene that has been mutagenized *in vitro*.

53. (Previously presented): The method of any of claims 50-52, wherein a marker gene is inserted into one or more of said mutagenized genes.

FINNEGAN  
HENDERSON  
FARABOW  
GARRETT &  
DUNNER LLP

1300 I Street, NW  
Washington, DC 20005  
202.408.4000  
Fax 202.408.4400  
www.finnegan.com

54. (Currently amended): A modified *Shigella* for use in making a vaccine against a wild strain of *Shigella*, the modified *Shigella* comprising:

(a) an inactivated *icsA* gene, inactivated by allelic exchange with a mutagenized *icsA* gene that has been mutagenized *in vitro*, wherein said mutagenesis is other than only by means of a transposon inserted into the gene; and

(b) an inactivated aerobactin or enterochelin gene, inactivated by allelic exchange with a mutagenized aerobactin or enterochelin gene that has been mutagenized *in vitro*, wherein said mutagenesis is other than only by means of a transposon inserted into the gene;

wherein the modified *Shigella* can not spread substantially within infected cells of the host, can not spread substantially from infected to uninfected cells of the host, and can not substantially invade cells of the host.

55. (Currently amended): The *Shigella* of claim 54, further comprising an inactivated *Shiga*-toxin gene, inactivated by allelic exchange with a mutagenized *Shiga*-toxin gene that has been mutagenized *in vitro*, wherein said mutagenesis is other than only by means of a transposon inserted into the gene;

wherein the modified *Shigella* can not spread substantially within infected cells of the host, can not spread substantially from infected to uninfected cells of the host, can not substantially invade cells of the host, and can not produce toxins that kill a substantial number of the host's cells.

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FARABOW  
GARRETT &  
DUNNER LLP

1300 I Street, NW  
Washington, DC 20005  
202.408.4000  
Fax 202.408.4400  
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56. (Previously presented): The *Shigella* of claim 54 or 55, wherein a marker gene is inserted into one or more of said mutagenized genes.

57. (Previously presented): A vaccine comprising the *Shigella* of claim 54 or 55 and a pharmaceutically acceptable vehicle.

Claims 58-73. (Cancelled)

74. (Currently amended): A modified *Shigella* for use in making a vaccine against a wild strain of *Shigella*, the modified *Shigella* comprising an inactivated *icsA* gene, inactivated other than only by means of a transposon inserted into the gene; wherein the modified *Shigella* can not spread substantially within infected cells of the host and can not spread substantially from infected to uninfected cells of the host.

75. (Previously presented): The *Shigella* of claim 74, wherein at least one nucleotide sequence has been deleted from said inactivated *icsA* gene.

76. (Previously presented): The *Shigella* of claim 74, wherein at least one nucleotide sequence has been inserted into said inactivated *icsA* gene.

77. (Previously presented): The *Shigella* of claim 76, wherein a marker gene is inserted into said inactivated *icsA* gene.

78. (Previously presented): A vaccine comprising the *Shigella* of claim 74 and a pharmaceutically acceptable vehicle.

79. (Currently amended): A modified *Shigella* for use in making a vaccine against a wild strain of *Shigella*, the modified *Shigella* comprising an inactivated *icsA* gene, inactivated by allelic exchange with a mutagenized *icsA* gene that has been mutagenized *in vitro*, wherein said mutagenesis is other than only by means of a transposon inserted into the gene;

wherein the modified *Shigella* can not spread substantially within infected cells of the host and can not spread substantially from infected to uninfected cells of the host.

80. (Previously presented): The *Shigella* of claim 79, wherein a marker gene is inserted into said mutagenized *icsA* gene.

81. (Previously presented): A vaccine comprising the *Shigella* of claim 79 and a pharmaceutically acceptable vehicle.

82-87. (Cancelled).

FINNEGAN  
ENDERSON  
FARABOW  
GARRETT &  
DUNNER LLP

1300 I Street, NW  
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Fax 202.408.4400  
www.finnegan.com